ISPH-0585
Docket No.: 30566/30021
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Bennett, *et al.*

Application No.: 09/918,186 Group Art Unit: 1635

Filed: July 30, 2001 Examiner: S. McGarry

For: ANTISENSE MODULATION OF SURVIVIN EXPRESSION

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia, 22313-1450

Sir:

I, Bharvin Patel, declare that:

1. I hold the degree of Doctor of Philosophy in Microbiology. I received my degree in 1991 from the Department of Medical Microbiology, St. George's Hospital and Medical School, University of London, London, UK. My Ph.D. thesis was entitled "Novel approaches towards the development of a rapid in-vitro viability and drug susceptibility assay for *Mycobacterium leprae*."

I am currently a Research Scientist and have been employed since 1998 by Eli Lilly and Company in Cancer research.

I have authored or co-authored 25 research papers published in scientific journals.

I am an inventor on 1 United States patent.

2. I further declare that the protocol design of following experiments, demonstrating that the antitumor activity of a compound of the invention described in the above-identified patent application Serial No. 09/918,186, were written by me and that the subsequent experiments were carried out by the in-vivo cancer research group of Eli Lilly and company.

Measurement of antitumor activity in a human glioblastoma xenograft tumor model

3. The compound examined for antitumor activity is the nucleic acid sequence of SEQ ID NO: 87, having phosphorothioate internucleoside linkages throughout the oligonucleotide, and having ten central 2'-deoxynucleotides flanked with four 2'-O-methoxyethyl (2'-MOE) nucleotides on the 5' and 3' ends of the oligonucleotide (hereinafter referred to as "23722"). Antitumor activity of 23722 was evaluated in a U-87MG human glioblastoma (Kiaris H, Schally AV, Varga JL, Antagonists of growth hormone-releasing hormone inhibit the growth of U-87MG human glioblastoma in nude mice Neoplasia. 2000 May-Jun;2(3):242-50.), and YUSAC-2, human melanoma (Grossman D, Kim PJ, Schechner JS, Altieri DC, Inhibition of melanoma tumor growth in vivo by survivin targeting. Proc Natl Acad Sci U S A. 2001 Jan 16;98(2):635-40) xenograft tumor models. A total of 10 CD1 nu/nu (Charles River) mice were used for each group. The Eli Lilly and Company Animal Care and Use Committee approved all the experimental protocols. For implantation, tumor cells were trypsinized, washed in PBS and resuspended in PBS at 6×10^7 cells/ml (U-87MG) and at 4×10^7 cells/ml (YUSAC-2) in DMEM. Just before implantation, animals were irradiated (450 TBI) and cells were mixed in matrigel (1:1). A total of 6×10^6 (U-87MG) and at 4×10^6 (YUSAC-2) tumor cells in a 0.2 ml volume were injected subcutaneously (s.c.) in the left rear flank. Treatment with 23722 (dissolved in 0.9% NaCl, injection grade), or mismatch control oligonucleotide (dissolved in 0.9% NaCl) or vehicle (0.9% NaCl) was started 3 days post tumor cell implantation. Compounds were administered intraperitoneally (i.p.) and intravenously (i.v.) for U-87MG and YUSAC-2 studies respectively in a 0.2 ml volume every other day for a total of 12 doses for the U-87MG study and 13 doses for the YUSAC-2 study. Tumor length and width were measured twice a week, and tumor volume was calculated using the formula: Tumor volume = $(L \times W^2) \times 0.536$. Tumor volumes were plotted against days post tumor implantation for each treatment group.

Results

4. As shown below, treatment with 25 mg/kg 23722 delayed human glioblastoma (Figure 1) and melanoma (Figure 2) tumor growth when compared with tumor bearing animals treated with vehicle or 25 mg/kg mismatch control oligonucleotide. This tumor growth delay due to 23722 treatment was statistically significant when compared to vehicle treatment group.

Figure 1.

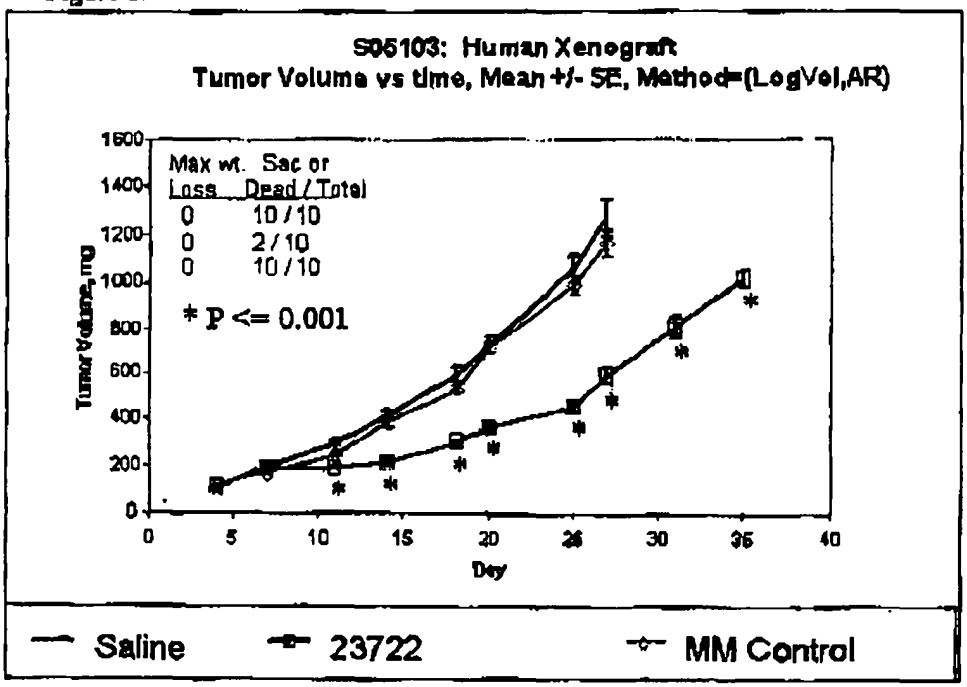
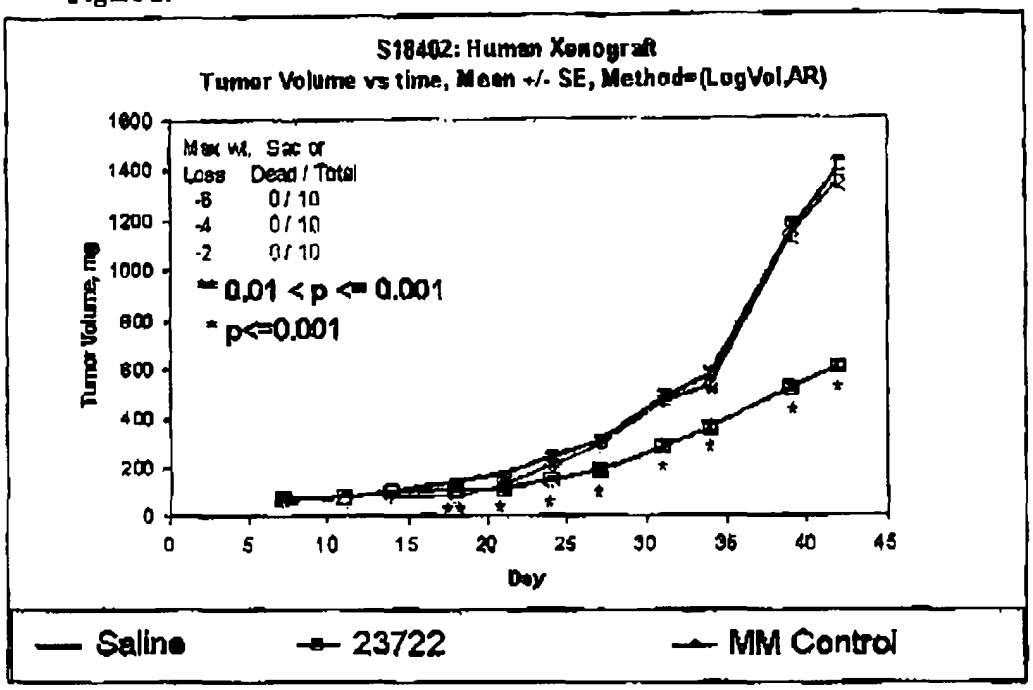


Figure 2:



Conclusion

5. Taken together, the results shown in Figures 1 and 2 demonstrate that the compound identified as 23722, described in patent application Serial No. 09/918,186, possesses activity as an *in vivo* inhibitor of tumor growth.

6. I further declare that all statements made herein of to the best of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of this application or any patent issuing thereon.

Bharvin K. R. Patel, Ph.D.

12/3/03